Remarks

The present application is directed to a method for detecting the presence of a target nucleic acid sequence by amplifying a target nucleic acid (wherein the target contains a purine-rich region or a purine-rich region is introduced into the target during amplification), adding a peptide nucleic acid (PNA), and detecting the presence of triplex structures. Claims 1-2, 5-6, 8-12, 14, 16, and 18-24 are pending in this application.

Applicants wish to thank the Examiner for withdrawing the rejections made in the previous Office Action under 35 U.S.C. §103(a).

Rejections under 35 U.S.C. §102

Claims 1-2, 5-6, 9, 12, and 22-24 have been rejected under 35 U.S.C. §102(b) as being anticipated by Seeger *et al.* (*Biotechniques*, 1997, 23(3):512-514, 516, 517). Applicants respectfully traverse the rejection.

The claims of the present application are directed to a method for detecting the presence of a target nucleic acid sequence in a sample by amplifying a target nucleic acid, (where the target nucleic acid contains a purine rich region or a purine-rich region is introduced into the target nucleic acid during amplification so the product has a purine-rich region), contacting the sample with a PNA molecule that binds to the target nucleic acid sequence, and detecting the presence of triplex structures. The detection of triplex structures indicates that the target nucleic acid sequence is present in the sample.

Seeger *et al.* disclose a **purification** process that uses PNA probes to remove genomic DNA from blood. In this process, biotin-labeled short bisPNA probes are bound to DNA in a

blood sample. The sample is then contacted with streptavidin-coated magnetic beads. The biotin (attached to the DNA via the PNA probes) binds to the streptavidin. Separation of the beads from the sample effectively pulls the DNA out of the sample. (See page 513 column 1, last paragraph.) Once separated, the beads are subjected to PCR to amplify a range of target genes found in the DNA. The PNA probes are designed to have a particularly low melting temperature so that they do not interfere with the PCR reaction (see page 514 column 1, lines 12-22). There is absolutely no suggestion that the amplified target genes will bind to the PNA probes. Furthermore, the PNA probes are used to separate the DNA from the blood and are **not** used to detect the amplification products. The PCR amplification products are detected entirely using conventional gel electrophoresis followed by staining with ethidium bromide (see page 513 column 3, lines 7-10). This applies even to the "test" case, described on page 514 column 1, where a plasmid engineered to incorporate a poly-A sequence is amplified.

Therefore, the process of Seeger *et al.* is fundamentally different from the present method in which PNA probes are included in a PCR reaction as a means of detecting the amplification product.

In addition, the Examiner asserts that the process of amplifying a target sequence having a purine-rich region is described in Seeger *et al.* The Examiner references page 216, however the Seeger *et al.* reference does not include this page number. Applicants respectfully request that the Examiner provide the correct page number for this statement. Applicants assume that the Examiner is referring to the "test" case on page 514, column 1,

where a plasmid has been engineered to incorporate a poly-A sequence, (which will hybridize to the PNA probe) and is amplified.

Applicants respectfully submit that step (b) of independent Claims 1 and 18 lacks a target nucleic acid sequence having a purine-rich region. In these claims, the purine-rich region is **introduced** during amplification. Seeger *et al.* fail to describe the introduction of a purine-rich region during amplification as claimed by applicants in Claims 1 and 18.

Furthermore, step (c) of independent Claims 1, 6 and 18 recites the detection of triplex structures where the detection of these structures indicates the presence of target nucleic acid in the sample. This detection step is not disclosed by Seeger *et al.*, who use gel electrophoresis and ethidium bromide staining to detect the amplification product, as shown in Figure 1 on page 514.

Contrary to the Examiner's assertion that Seeger *et al.* teach detection of triplexes (citing page 513, column 3, lines 5-10), applicants respectfully submit that this section merely describes an isolated experiment to determine the melting point of a triplex formed by bisDNA T7/DNA A7. The results demonstrate that the PNA probe does not interfere with the PCR reaction. Seeger *et al.* fail to suggest that the triplex structure can be used to detect amplification product. Although Seeger *et al.* mention triplexes, it is not in the same context and there is no suggestion that triplexes could form the basis for detection of amplification product (see page 513, column 3, Results section). Seeger *et al.* certainly fail to disclose triplex detection after or during an amplification reaction as required by the claims. If one looks at Figure 1 on page 514 of Seeger *et al.*, it appears that no triplex structures are present in the amplification product because the bands on the gel appear to be at the same distance

along the gel, irrespective of whether PNA probe was present or absent in the PCR reaction. If the product included triplexes, such triplexes would have a higher molecular weight and would run more slowly down the gel as compared with product lacking triplexes.

Thus, the claimed method is clearly different from the method of Seeger *et al.* and therefore novel.

Rejections under 35 U.S.C. §103

Claims 14-16 and 18-21 have been rejected under 35 U.S.C. §103(a) as obvious over Seeger *et al.* (*Biotechniques*, 1997, 23(3):512-514, 516, 517) in view of Felgner *et al.* (U.S. Patent No. 6,165,720). Applicants respectfully traverse the rejection.

The Examiner asserts that Seeger *et al.*, described above, shows introduction of purine rich regions by way of primers which contain purine residues, citing page 513, column 2, paragraphs 3-5.

Applicants respectfully submit that the PNA primers of Seeger *et al.* are designed to match the target gene. Therefore, their structure is determined entirely by the preexisting gene sequence. They do not introduce any additional purines into the target. In addition, the results shown in Figure 1 suggest that the triplexes could not be utilized as a detection means because there in no evidence that they exist in the amplification product.

The Examiner also asserts that Felgner *et al.* teach detection of a fluorescent label using a surface plasmon resonance detector. Applicants respectfully submit that Felgner *et al.* fails to disclose the use of a surface plasmon resonance detector for detecting PNA triplexes. Applicants can find no reference in Felgner *et al.* to surface plasmon resonance or wave guides or anything of the sort. The passage cited by the Examiner (column 14, lines 21-

58) discusses detection using a FRET analysis conducted in solution using a spectrofluorimeter. There is no suggestion at all that the PNA is immobilized on anything, and certainly not a surface plasmon resonance detector.

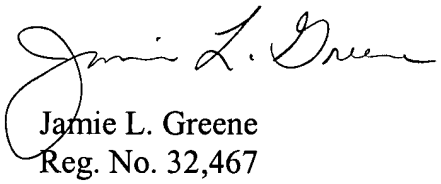
As discussed above, the Seeger *et al.* paper is deficient in disclosing the claimed method and Felgner *et al.* fails to make up for these deficiencies. Neither reference alone or in combination discloses all of the claimed elements to arrive at the claimed invention. Applicants respectfully request withdrawal of this rejection.

Conclusion

Applicants submit that the pending claims define novel and patentable subject matter and provide a complete response to the Office Action. Accordingly, Applicants respectfully request allowance of these claims. No additional fees are believed due, however, the Commissioner is hereby authorized to charge any deficiencies which may be required, or credit any overpayment, to Deposit Account Number 11-0855.

Early and favorable consideration is earnestly solicited. If the Examiner believes any informalities remain in the application that can be resolved by telephone interview, a telephone call to the undersigned attorney is earnestly solicited.

Respectfully submitted,



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